Long-term cardiovascular outcome of renal transplant recipients after early conversion to everolimus compared to calcineurin inhibition

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Abstract

Long-term data on cardiovascular (CV) outcome of renal transplant recipients (RTR) on mTOR-i (mammalian Target Of Rapamycin-inhibitors) are scarce. In a sub-study of the MECANO trial we investigated changes in intima media thickness (IMT), CV risk profile, Major Adverse CV Events (MACE) and survival in RTR on a mTORi versus CNI based regimen.

Patients (enrolled 361) were treated with (basiliximab) and triple IS (CsA-Cyclosporine A-(C), MPS(M), prednisolone(P)). At M6 patients were randomized (n=224) to the CsA group (C, P, N=89), MPS group (M, P, N=39) EVL group (Everolimus, P, N=96).

At week 2, M6 and M 24, IMT measurements of the Common Carotid Artery were performed. Cardiovascular risk factors were assessed at baseline, 6 and 24 months of follow-up. Seven years survival and MACE -free survival probability were calculated by the Cardiovascular Risk Calculator for RTR. After seven years of follow-up incidence of cardiovascular events and patient survival were assessed. Mean IMT at baseline, (N=192), was 0.64±0.14 mm. At M6 (N=158), 0.66±0.15, M24 IMT was 0.68±0.15 (N=95).

Conclusion: No significant differences between groups concerning IMT, true CV events and mortality, CV risk profile, predicted MACE / Mortality were found between mTORi and CNI-based regimen after 7 years of follow-up.

Introduction

Chronic kidney disease is a major worldwide public health problem. Renal transplantation has been established as the optimal treatment for end stage renal disease. Despite this, after renal transplantation long-term life expectancy is limited, which is mostly due to the increased risk of cardiovascular (CV) disease[1].

The introduction of Cyclosporine A (CsA) and other Calcineurin inhibitors (CNI's) significantly improved the outcome of all solid-organ transplants by reducing the risk of rejection.[2] Nevertheless, long term calcineurin inhibitor (CNI)-based immunosuppression is associated with nephrotoxicity and other adverse events, including hypertension, hyperlipidemia and diabetes mellitus[3]. Therefore, CNI- sparing regimens have been proposed to improve graft function and cardiovascular outcome after renal transplantation.

Multiple randomized controlled trials (RCT) have investigated mTOR inhibitors as a potential alternative for CNIs, overall resulting in better renal function, but at cost of increased risk of rejection[4,5].

However, long term data on outcome, and especially CV outcome in renal transplant recipients (RTR) on mTORi are scarce.

In the present study, as a sub-study of the MECANO trial, IMT and change of IMT over time were prospectively measured as a CV outcome parameter in RTR. Additionally, we assessed cardiovascular risk profile, and compared true long-term CV event-free survival and mortality in RTR randomized to a mTORi- versus CNI-based immunosuppressive regimenFinally, we aimed to reproduce the previously published cardiovascular risk calculator from Soveri et al[6]

Patients and methods

Between November 2005 and June 2009 361 de novo kidney transplant patients were recruited in three Dutch Transplantation centers to participate in the MECANO trial. The study was conducted according to the Good Clinical Practice guidelines and in accordance to the ethical principles of the declaration of Helsinki and was approved by the Dutch Medical Ethical Board for medical research.[5] (Trial registration NTR1615). All patients gave written informed consent. This study was a 24-month, prospective, multi-center, open-label randomized controlled trial, aiming at optimizing immunosuppression and reducing side effects, including cardiovascular outcome. The quadruple immunosuppressive regimen for all patients during the first six months was similar: induction with basiliximab, followed by CsA, MPS and prednisolone[7]. At month six a protocol biopsy was performed. When no histological signs of rejection were seen, patients were randomized to receive dual immunosuppressive therapy with either CsA, MPS or Everolimus, all in combination with prednisolone. In case of (borderline) rejection patients were not randomized. For detailed description see Bemelman et al.[5] Primary endpoint of the MECANO study was the development of interstitial fibrosis at the 24-month protocol biopsy[5], published in AJT 2016, secondary end-points were amongst others CV endpoints; Intima Media Thickness (IMT) of the common carotid artery, blood pressure and the number of antihypertensives, lipid profile, fasting glucose and HbA1C. The present study reports mentioned secondary endpoints.

After enrollment of 39 patients, the MPS -arm was prematurely stopped by the Data Safety Monitoring Board, because of an unacceptably high rejection percentage (21%). Patients in the MPS-arm stayed on MPS when they did well and completed the trial (n=39), the trial continued as a two-armed trial, comparing CsA and EVL.

Intima Media-Thickness measurement was performed at approximately day 14 (W2), after six months (M6) and after 24 months (M24). The longitudinal axis of the common carotid artery was scanned in the supine position, at the right and left side. Acuson 128XP systems (Acuson Corporation, Mountain View, CA, USA) with 7.0 MHz ultrasound transducer were used in all 3 performing units. All measurements were performed by trained sonographers.

Subsequently, all measurements were scored and verified by 2 persons, the inter-observer variation was 3.9 %.

For scoring cardiovascular risk we used the previously described Cardiovascular Risk calculator for renal transplant recipients[6]. Additionally, cardiovascular events and mortality data were collected until 7 years after renal transplantation.

Statistical analysis

To test whether variables were normally distributed, we used the Kolmogorov-Smirnov test. Multiple group comparisons were computed with the Kruskal-Wallis Test. To test effects of time point (visit), the multivariate Friedman test was used. To compare two time points, Wilcoxon Signed Ranks test was used. We calculated ROC-curves using the 7-years events and deaths, as previously poublished by Soveri.

Since the distribution of pMACE and pMort was not normal, we transformed these variables to wMACE and wMort, using the logit transformation[8,9] , before using the variables in a regression analysis. Survival was assessed by Kaplan-Meier estimates for survival distribution. Differences between groups in survival were analysed with log-rank tests. Statistic tests were performed using SPSS 22.0 (2013, IBM Corp., Armonk, NY, USA).

Linear mixed model analyses were performed using Stata 14 (2015, StataCorp LP., College Station, Texas, USA). We performed all analyses twice: first comparing IMT in the MPS vs. CsA vs. EVL groups. Second, we compared IMT in the CsA versus EVL groups. We assigned the intercept and slope of the individuals and the medical center of treatment as random factors. A scaled identity covariance structure was applied and we assigned all predictors as fixed factors. The interaction (calculated as the product) of the study group and follow-up time in months (from the first IMT assessment at week 2) was regarded as the slope of IMT per group over follow-up time. We adjusted the associations for sex and age (model 1) and consequently smoking behavior (never, ex or current) and BMI (model 2).

P-values below 0.05 were considered significant.

Results

In total 224 patients were randomized after renal transplantation. Of these patients, 89 were randomized to the CsA group, 39 to the prematurely stopped MPS- and 96 to the Everolimus group. Patient characteristics did not differ significantly between the three groups (Table 1).

In total 119 (53%) patients received a kidney from a living donor, 67 (30%) of a DBD (donation after brain death), and 37 (17%) of a DCD (donation after cardiac death) donor. Mean age at time of transplantation was 51.6, mean donor-age 47.1 years. For those patients receiving dialysis, mean total time on renal replacement therapy (TTRRT) was 32 months. Half of all patients never smoked, 33.6 % were previous smokers and 16.3 % was still smoking.

Intima Media Thickness

The first IMT in this study was assessed at W2 (n=192) and the mean in all patients was 0.64 ±0.14 mm. (Fig 1). All IMT measurements are also shown in Table 2. At randomization (6 months), the mean IMT in all patients (n=158) was 0.66 ± 0.15 mm. The IMT at six months after renal transplantation did not differ significantly between the three treatment groups. At 24 months, IMT was 0.66 (±0.14) mm in the CsA group and 0.66 (±0.13) mm in the EVL group, whereas it was 0.76 (± 0.2) mm in the MPS group.(p=0.06) For analysis of change in IMT over time, we only used the assessments of 95 patients that had an IMT measurement at 24 months. For all these patients measurements were available at 2 weeks and 6 months after transplantation (Fig 2). No significant differences were observed in change of IMT over time after renal transplantation.

We also analyzed subgroups of patients for differences in IMT change. Neither RTR transplanted with a living versus deceased donor, nor patients who were transplanted pre-emptively versus those transplanted after start of renal replacement therapy, showed significant differences in change of IMT over time.

Patients who developed a CV event during the study (24) and in whom IMT was measured (n = 23), did not have significant higher IMT at baseline as compared to patients who did not suffer from a CV event. (p=0.26). Also, the change in IMT – a decrease of 0.03 mm in patients suffering of a cardiovascular event (n=11) and an increase of 0.06 mm in patients who died (n=10) - was not significantly higher, than in patients who did not develop a CV-event.

Finally, we performed a mixed model analysis comparing IMT. In the first analysis we compared the CSA, EVL and MPS groups: no significant association was found between treatment arm and IMT slope over follow-up time (supplementary Table S1: β [95%CI]: 0.0001 [-0.003–0.003], CsA: p = 0.96; EVL: -0.0004 [-0.0003–0.003], p=0.78, MPS: reference. Adjusted for age, sex, BMI, and smoking behavior). A significant positive association was found of age (β [95%CI]: 0.005 [0.004–0.007], (p <0.001) and a history of smoking (β [95%CI]: 0.04 [0.003–0.07], (p = 0.03), with mean IMT over the follow-up period. In the second analysis we compared CSA and EVL groups but no significant differences were found between these two treatment groups in IMT slope (supplementary Table S2: EVL: β [95%CI]: -0.0004 [-0.002–0.002], p = 0.69; EVL: reference. Adjusted for age, sex BMI, and smoking behavior). Similarly, age (β [95%CI]: 0.005 [0.004–0.006], p<0.001) and a history of smoking (β [95%CI]: 0.06 [0.03–0.09], p = 0.001) were associated with mean IMT over time.

Cardiovascular risk factors

Systolic blood pressure, as well as diastolic blood pressure in all groups remained stable, no significant differences were found between the three groups at any moment.

The total number of antihypertensive (AH) agents decreased immediately after transplantation, but increased towards 6 months, and at 2 years after transplantation the number of prescribed AH was 2.1 (mean), but this was not significantly more than at two weeks after renal transplantation, and did not differ significantly between the three treatment groups. Over time between groups diuretic use did not differ significantly (table 2).

BMI increased significantly in all patients both from week two (25.2) to month 6 (26.1) and again from month 6 to month 24 (26.9). However, between the groups, no significant differences were found.

Also, HbA1c values increased significantly over time, but did not differ between the randomization groups. In concordance herewith, fasting glucose levels increased over time and were significantly ($p<0.05$) higher at 24 months after renal transplantation than all previous measurements for all patients. Again, no differences between the three groups were found.

For total-cholesterol levels, a significant ($p< 0.005$) increase over the total cohort was found. These levels increased from 4.2 mmol/l at start to 5.1 mmol/l at 24 months after transplantation. However, LDL cholesterol levels did not change over time. In both total- and LDL-cholesterol levels no differences were found between the 3 groups. The use of statins increased significantly from 44.8% at baseline, towards in 68.8 % at 24 months in all patients.

Cardiovascular events and mortality

The chance to suffer from a major adverse cardiac event was predicted by the previously validated pMACE score. The pMACE score did not differ between the groups and was 10% in the CsA group, 11% in the MPS and 12% in the EVL-group.

After 7 years of follow-up a CV event occurred in 11% of the patients of CsA group, 16% in the MPS group and 10% in the EVL group , and did not differ significantly between groups ($p=0.58$). Patients with a CV event had a higher pMACE than patients without (0.15 vs. 0.10, $p=0.009$). IMT and transplant function were not different between patients with and without a CV event. (IMT: $p=0.26$, MDRD: $p=0.41$).

The predicted chance to die within 7 years was 13, 15 and 15% respectively. Overall, nine patients (10%) died in the CsA group, six patients (15%) in the MPS and 16 (17%) in the EVL group. (($p= 0.42$) Table 3). Patients who died had a higher pMort than patients who did not (0.13 vs. 0.22, $p<0.001$). Of the 31 patients who died within 7 years, four had a cardiovascular event, 25 did not and in two patients the cause of death was unknown. However, in 60 % of patients who died the cause of death was registered as “not determined”, so a cardiovascular cause cannot be fully excluded. Eventually, both the incidence of cardiovascular events and mortality rate did not differ significantly between the three treatment groups . (Fig 3)

Replication ROC-curves of Soveri et. al.[6]

We calculated ROC-curves using the 7-year events and deaths (Figure 4). These values correspond to previous results performed by Soveri et al.

The area under the curve for MACE was 0.70 (95% confidence interval 0.534-0.860). For mortality, the area was 0.77 (95% confidence interval 0. 678-0.863).

Discussion

It is well established that renal transplantation is the optimal treatment option for patients with renal insufficiency. However, also after renal transplantation the risk of cardiovascular events and the overall mortality is increased as compared to the population as a whole. It is hypothesized that the choice of immunosuppressive drug might influence the long-term outcome. However, the results of our study show that the long-term cardiovascular risk as evaluated by CV risk factors, IMT (as a marker of CV disease), calculated risk of CV events (pMACE) and the occurrence of CV events up to seven years after RT , did not differ in kidney transplant recipients on mTORi or CsA.

Scarce evidence is available concerning kidney transplantation and the alleged profit of mTORi in relation to long-term outcome. As shown in 2005, kidney transplant patients with poor renal function have a higher risk of all- cause and CV death[10]. The Five-year outcomes after conversion from CsA to Everolimus; the randomized ZEUS study[11] showed a significant improvement in renal function that is maintained to at least 5 years. In a systematic review and meta-analysis of individual patient data by Knoll et al a higher mortality rate was found in renal transplant recipients (RTR) on the mTORi sirolimus.[12] Also, a retrospective analysis of the United States Renal Data System (USRDS) by Isakova et al[13] found a higher mortality rate in association with sirolimus. However, these registry data should be interpreted with care due to, among others, temporal trends and indication bias. Regarding everolimus, a recent long-term study by Lim et al combining everolimus with reduced CsA versus mycophenolate sodium with standard CsA did not find differences in mortality rate or graft loss between the study arms [14].

Additionally, Knoll et al reported higher mortality rates with higher everolimus trough levels (defined > 10 ng/ml). Also, in our study mean trough levels were higher than in more recent CNI- conversion or CNI-combination studies, which might explain higher discontinuation rates.

At the time of designing the present study, Intima Media Thickness was considered the best non-invasive sonographic marker for early atherosclerosis vascular wall lesions[15]. In a community-based cohort study it was found that impairment in kidney function was associated with adverse changes in arterial structure in a general elderly population. These changes occurred in an early stage of deterioration of kidney function, and they were predictors of cardiovascular outcome[16].

In a previous study in a cohort with early stage chronic kidney disease, a median IMT of 0.6 mm (0.4-0.7 mm) was found[17].

A comparison of IMT in dialysis and kidney transplant recipients patients showed that IMT in dialysis patients was significantly higher compared to kidney transplant recipients, and IMT increased with longer duration of dialysis[18].

Due to beneficial results with Everolimus, amongst others seen in heart transplantation[19], we expected a slower increase in IMT in the EVL group. IMT in our study, however, did not

differ significantly between groups, nor did it change over time. No association was found with the occurrence of cardiovascular events during long-term follow-up. Additional analyses by mixed models showed associations of IMT with age and smoking status but not with immunosuppressive therapy. In conclusion, IMT measurement was not of added value in detection of patients at increased risk for cardiovascular events after renal transplantation. That result seems to be inconsistent with earlier findings in both the general population and CKD patients.[20]

In another study in which 17 patients were converted from CsA to everolimus, Pulse Wave Velocity increased significantly in the 10 patients who continued CsA group between 6 and 15 months, whereas a slight decrease (ns) was found in the Everolimus group.[21] However, recently the 2 years results from the ELEVATE study were published and suggested that conversion from CNI to everolimus at 10-14 weeks after kidney transplantation is not associated with ventricular mass index and pulse wave velocity.[22] So currently available evidence does not suggest clinically relevant differences between CNI and mTORi on these cardiovascular endpoints in renal transplant recipients.

Regarding the cardiovascular risk profile the patients included in our trial had an elevated blood pressure, cholesterol and glucose-level. Although statin use increased from 45% to 69 % of the patients LDL cholesterol did not reach target levels < 2.5 mmol/L. Nowadays statins are advised by protocol for all recipients of renal transplantation (KDIGO Guideline for the Care of Kidney Transplant Recipients, Dyslipidemia).[23] Between the CsA and EVL groups no significant differences were found in cardiovascular risk profile. Also, the ELEVATE study in which cardiovascular parameters were studied in patients after an early switch to everolimus (mean trough level of everolimus throughout the study 7.2 ng/mL) or remaining on CNI, showed no significant difference in cardiovascular parameters, nor in mortality rate [22,24]. And, in a study from Spagnoletti, in which patients were assigned to either tacrolimus/MMF, or Everolimus/ low CsA, cardiovascular risk profiles were similar, although the latter group showed significantly higher dyslipidemia.[25] In this study the use of antihypertensive agents was similar between CsA- and TAC-based regimen. However, although the number of deaths in our study due to cardiovascular causes was low, it cannot be excluded that these were underreported in the registry. Nevertheless, our study results emphasize the importance of adequate cardiovascular risk management in this high risk population.

The Cardiovascular Risk calculator for renal transplant recipients was published in 2012, and we were able to evaluate the occurrence of CV events and death 7 years after renal transplantation, thereby reproducing the Risk calculator predictions. In our study with 7 years follow-up the incidence of cardio vascular events and mortality was high, as expected in this high risk population. However, no difference between mTORi and CNI was found. In the ALERT study from which the Cardiovascular Risk calculator was derived the majority of patients used prednisolone and/or azathioprine. As only a small subgroup used modern immunosuppressive drugs as CNI, mTORi and mycophenolic acid, thus far it was not clear whether this tool was able to predict mortality or CV events dependent on the choice of immunosuppression. Hereafter, MACE and mortality risk calculator have been externally validated and found suitable in the BENEFIT and BENEFIT-EXT trials, including belatacept and CsA treated patients[26]. Now, the ROC-curves in the current study including mTORi treated patients were comparable to the curves of the group of Soveri et al. And therefore,

this cardiovascular risk calculator seems to predict cardiovascular risk independent of the choice of immunosuppressive drug.

This study shows that in this fairly low risk cohort of Dutch patients the use of Everolimus is neither superior nor inferior to the use of CsA, in relation to CV outcome, so both regimes can be used safely.The current study had several limitations. The MPS arm was stopped prematurely, so limited data for this group were available. Secondly, between performing IMT assessments and actual scoring and analysis of IMT-data a long time passed by due to several and various personnel- and software problems. Finally, between IMT measurement at 24 months and subsequent follow-up a period of max 5 years could have been passed. This could have led to disparity between the IMT measurements and the occurrence of these events. Additionally, due to technical failure, in one of the centers at 24 months no appropriate measurements of IMT were performed. Therefore IMT- data at 24 months were limited to two centers. Due to study design only CsA was included, and Tacrolimus-based immunosuppression was not evaluated. Obviously, this could have impacted the outcomes of our study on cardiovascular events and especially hypertension, as CsA is associated with different side effects as compared to tacrolimus. Currently, CNIs are also more often combined with mTORi, however, this regimen was not studied in this RCT. Furthermore, the endpoint of the MECANO study was powered on renal function, CV outcome was a secondary endpoint. Finally, the study size was limited and after seven years only a limited number of patients used the initial immunosuppressive regimen, resulting in a limited sensitivity for detecting differences in the occurrence of cardiovascular events between the treatment groups.

In conclusion:

In depth, cardiovascular risk profiling by the measurement of IMT, and traditional cardiovascular risk factors, as well as long-term follow-up over a period of seven years of CV events, this study did not show significant differences in any CV surrogate or true endpoints between RTR on prednisolone with CsA, with MPS or with EVL. Therefore, based on CV risk profile, choice of immunosuppressive regimen seems not warranted. Additionally, we conclude that measurement of IMT did not contribute to CV-risk profiling, whereas pMACE predicted the occurrence of cardiovascular events. Further studies and adherence to current cardiovascular guidelines are needed to further improve CV outcome of this high risk population.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by Transplant International.

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Marja van Dijk: Performed research, collected data, analyzed data, drafted manuscript

Arie M. van Roon: Analyzed data and helped with manuscript

M. Yusof Said: Analyzed data (Mixed models)

Frederike J. Bemelman: Designed research, participated in patient care

Jaap J. Homan van der Heide: Designed research, participated in patient care

Hans W. de Fijter: Designed research, participated in patient care

Aiko P.J. de Vries: Participated in patient care

Stephan J.L. Bakker: Revised the manuscript

Jan Stephan F. Sanders : Edited the manuscript

Table 1: Baseline characteristics of randomized patients

361 patients were enrolled, but 137 did not continue on assigned treatment, so 224 randomized patients remained.

Variable, mean (sd) or %	*CsA/P*	*MPS/P*	*EVL/P*	*Total*
Number of randomized patients	89	39	96	224
Age (yr)	49.7 (12.7)	53.7 (11.3)	51.4 (12.8)	51.1 (12.5)
Sex (% Male)	62.9	64.1	64.6	63.8
First transplant (%)	95.5	92.3	93.8	94.2
Cause (%)				
Diabetes mellitus	2.2	5.1	4.2	3.6
Hypertension	14.6	23.1	15.6	16.5
Glomerulonephritis	19.1	12.8	17.7	17.4
Pyelonephritis or interstitial nephritis	3.4	0.0	3.1	2.7
Focal segmental glomerulosclerosis	3.4	5.1	4.2	4.0
Urologic	5.6	5.1	10.4	7.6
Polycystic Kidney Disease	23.6	23.1	20.8	22.3
Vascular	4.5	5.1	5.2	4.9
Other	23.6	20.5	18.8	21.0
Donor type (%)				
Deceased after brain death	25.8	41.0	29.5	30.0
Deceased after cardiac death	14.6	17.9	17.9	16.6
Living related	25.8	15.4	22.1	22.4
Living unrelated	33.7	25.6	30.5	30.9
Donor age (yr)	48.7 (13.7)	43.4 (15.8)	49.3 (12.8)	48.1 (13.8)
Antigen mismatch (n)	2.8 (1.5)	2.8 (1.8)	2.9 (1.5)	2.8 (1.6)
Cold ischemia time (hr)				
Living	2.4 (0.6)	2.4 (0.6)	2.8 (2.3)	2.6 (1.5)
Deceased	17.2 (4.8)	16.1 (5.6)	15.6 (5.2)	16.3 (5.2)
TTRRT (month)	27.7 (28.9)	33.4 (31.1)	35 (33.5)	31.8 (31.4)
Smoking (%)				
Never	48.3	48.7	53.1	50.4
Previous	34.8	35.9	30.2	33.0
Current	16.9	15.4	16.7	16.5

TTRRT=Total Time on Renal Replacement Therapy. No statistical significant group differences were found.

Table 2: Outcome parameters

Group	*CsA/P*		*MPS/P*		*EVL/P*		p_{Groups}	*Total*	
Variable	N	mean (sd)	N	mean (sd)	N	mean (sd)		N	mean (sd)
IMT [mm]									
At wk 2	81	0.62 (0.12)	30	0.71 (0.19)	81	0.64 (0.14)	0.12	192	0.64 (0.14)
At mo 6	69	0.64 (0.15)	22	0.72 (0.22)	67	0.65 (0.12)	0.35	158	0.66 (0.15)
At mo 24	39	0.66 (0.14)	17	0.76 (0.20)	39	0.66 (0.13)	0.06	95	0.68 (0.15)
SBP [mmHg]									
At wk 2	77	146 (20)	28	143 (18)	79	143 (19)	0.56	184	144 (19)
At mo 6	80	141 (18)	30	143 (26)	76	146 (19)	0.11	186	143 (20)
At mo 24	67	143 (22)	24	146 (20)	64	140 (17)	0.37	155	142 (20)
DBP [mmHg]									
At wk 2	77	85 (11)	28	82 (9)	79	84 (12)	0.41	184	84 (11)
At mo 6	80	84 (11)	30	82 (13)	76	86 (13)	0.25	186	84 (12)
At mo 24	67	85 (12)	24	81 (12)	64	81 (10)	0.10	155	83 (11)
BMI [kg/cm²]									
At wk 2	76	24.9 (3.7)	28	26.2 (3.6)	80	25.2 (3.8)	0.33	184	25.2 (3.7)
At mo 6	76	26.0 (3.8)	30	26.1 (3.8)	79	26.2 (3.8)	0.95	185	26.1 (3.8)**

At mo 24	64	26.9 (4.4)	21	27.0 (5.2)	65	26.9 (3.8)	0.95	150	26.9 (4.3)** ##
Creatinine [µmol/l]									
At mo 6	81	125.0 (31.8)	30	112.4 (37.4)	81	125.9 (40.6)	0.19	192	123.4 (36.7)
At mo 24	78	140.6 (46.2)	29	120.4 (43.7)	81	138.2 (59.6)	0.13	188	136.4(52.3)**
HbA1c [mmol/mol]									
At baseline	73	43.0 (11.8)	27	43.2 (10.2)	71	41.1 (9.9)	0.66	171	42.3 (10.8)
At wk 2	71	42.5 (10.7)	29	41.9 (9.0)	74	40.2 (8.9)	0.48	174	41.4 (9.7)‡
At mo 6	76	42.5 (11.7)	30	47.7(17.6)	77	42.5 (9.2)	0.51	183	43.4 (12)
At mo 24	67	42.9 (9.7)	24	44.7(11.9)	69	44.7 (9.4)	0.34	160	44.0 (9.9)#
Glucose [mmol/l]									
At baseline	74	5.3 (1.2)	24	5.9 (2.0)	72	5.3 (1.0)	0.43	167	5.4 (1.3)
At wk 2	72	5.3 (1.3)	25	6.5 (5.3)	72	5.5 (1.7)	0.53	169	5.6 (2.5)
At mo 6	76	5.5 (1.6)	25	6.2 (3.2)	74	5.5 (1.7)	0.35	175	5.6 (1.9)
At mo 24	68	5.6 (1.7)	25	6.2 (2.5)	68	6.2 (2.2)	0.12	161	6.0 (2.1)‡
Cholesterol [mmol/l]									
At baseline	73	4.3 (1.2)	22	3.9 (0.7)	71	4.3 (1.0)	0.38	167	4.2 (1.1)
At mo 6	78	5.2 (1.0)	30	4.8 (0.8)	80	5.1 (1.0)	0.11	188	5.1 (1.0)**
At mo 24	69	5.1 (1.0)	25	4.8 (1.0)	71	5.3 (1.1)	0.08	165	5.1 (1.1)##

LDL [mmol/l]									
At mo 6	79	3.1 (0.9)	27	2.8 (0.8)	80	3.1 (0.9)	0.17	186	3.1 (0.9)
At mo 24	68	2.9 (0.8)	25	2.8 (0.8)	71	3.0 (0.9)	0.49	164	3.0 (0.9)*
Anti-hypertensives									
At baseline	81	2.07 (1.37)	30	1.53 (1.33)	81	1.98 (1.15)	0.10	192	1.95 (1.28)
At wk 2	81	1.42 (0.96)	30	1.23 (0.86)	81	1.30 (0.86)	0.70	192	1.34 (0.90)**
At mo 6	81	1.80 (0.98)	30	1.77 (0.82)	81	1.98 (1.01)	0.42	192	1.87 (0.97)**
At mo 24	81	2.11 (1.08)	30	1.77 (0.94)	81	2.16 (1.10)	0.16	192	2.08 (1.07)**
Statin use		percentage		percentage		percentage			percentage
At baseline	81	43.2%	30	50.0%	81	44.4%	0.81	192	44.8%
At mo 6	81	61.7%	30	53.3%	81	54.3%	0.57	192	57.3%**
At mo 24	81	74.1%	30	50.0%	81	70.4%	0.05	192	68.8%+##
Diuretics use		percentage		percentage		percentage			percentage
At baseline	72	52.8%	26	88.5%	67	55.2%	0.004	165	59.4%
At wk 2	72	84.7%	26	88.5%	67	80.6%	0.62	165	83.6%**
At mo 6	72	75.0%	26	73.1%	67	65.7%	0.47	165	70.9%*#
At mo 24	72	70.8%	26	69.2%	67	55.2%	0.14	165	64.2%*

** p<0.05 significant change from previous time point, ** p<0.005 significant change from previous time point*

# p<0.05 significant change from baseline, ## p<0.005 significant change from first time point

‡ p<0.05 significant change from all other time points

p_{Groups}: p-value for test of group differences (Kruskal Wallis test)

IMT: Intima Media Thickness

SBP: Systolic Blood Pressure

DBP: Diastolic Blood Pressure

LDL: Low Density Lipoprotein

To test the variables if they are normally distributed, we used the Kolmogorov-Smirnov test. Group comparisons are tested with the Kruskal-Wallis Test. To test effects of time point (visit), we used the Friedman test. To compare two time points, we used Wilcoxon Signed Ranks test. Tests with p-values below 0.05 are considered significant.

Table 3: Events at 7 years post TX and risk scores at baseline

Event, risk	*CsA/P*	*MPS/P*	*EVL/P*	*Total*	*P-value*
Number of CV events (%)	9 (11)	6 (16)	9 (10)	24 (11)	0.58
Number of death (%)	9 (10)	6 (15)	16 (17)	31 (14)	0.30
pMACE06 (max)	0.10 (0.47)	0.11 (0.32)	0.12 (0.45)	0.11 (0.47)	0.47
pMort06 (max)	0.13 (0.39)	0.15 (0.56)	0.15 (0.44)	0.14 (0.56)	0.28

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Figure 1: Outcome IMT

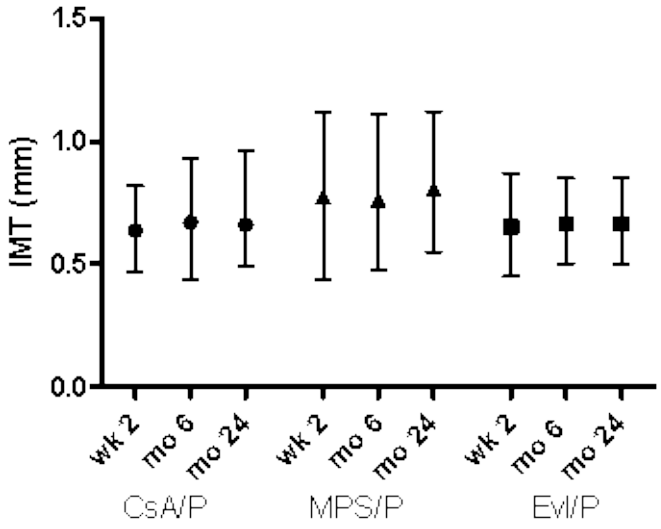


Fig 2: CONSORT diagram Patient flow and numbers of IMT- measurements

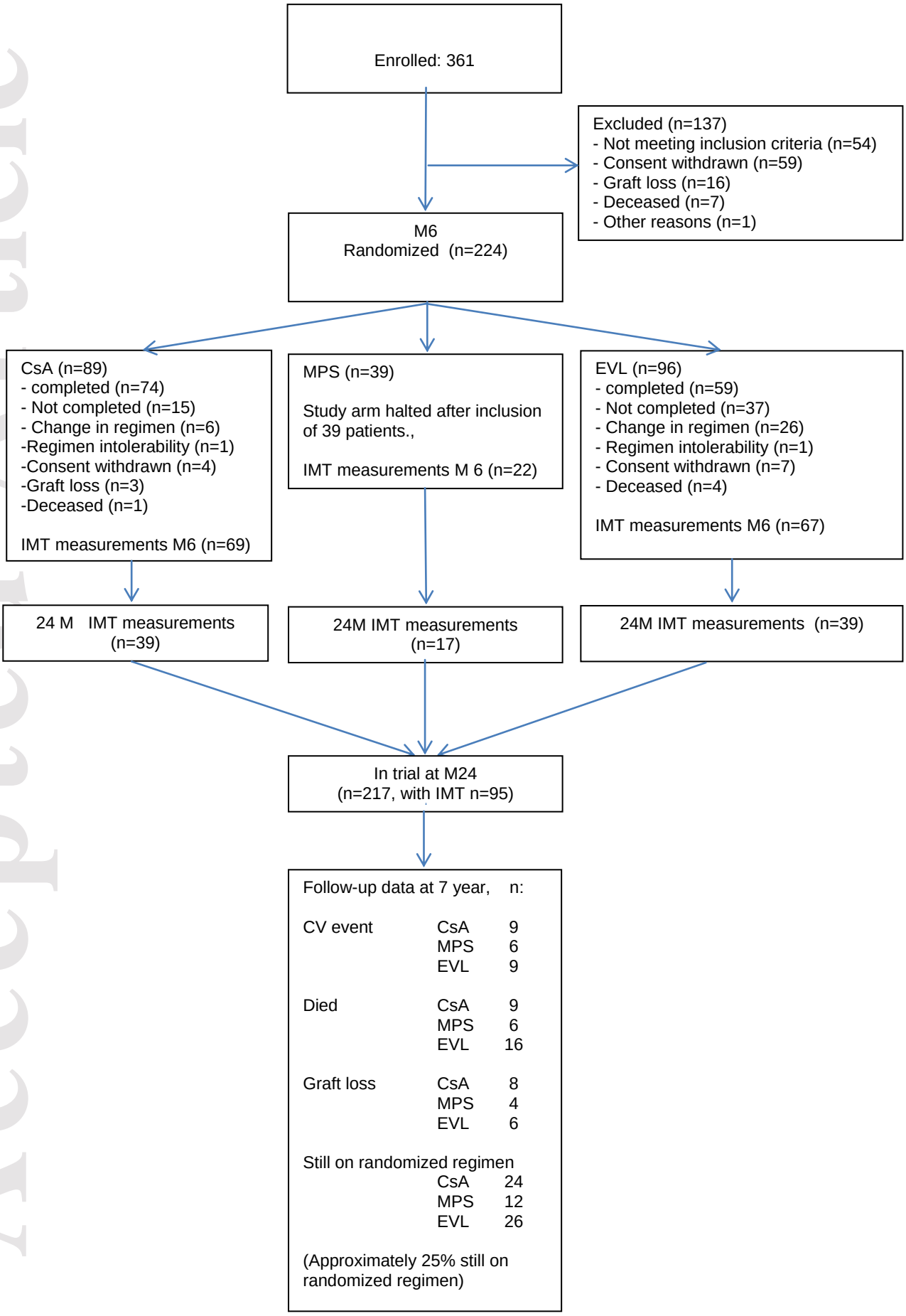


Figure 3: Survival in years. Log-rank test for group differences: p=0.286.

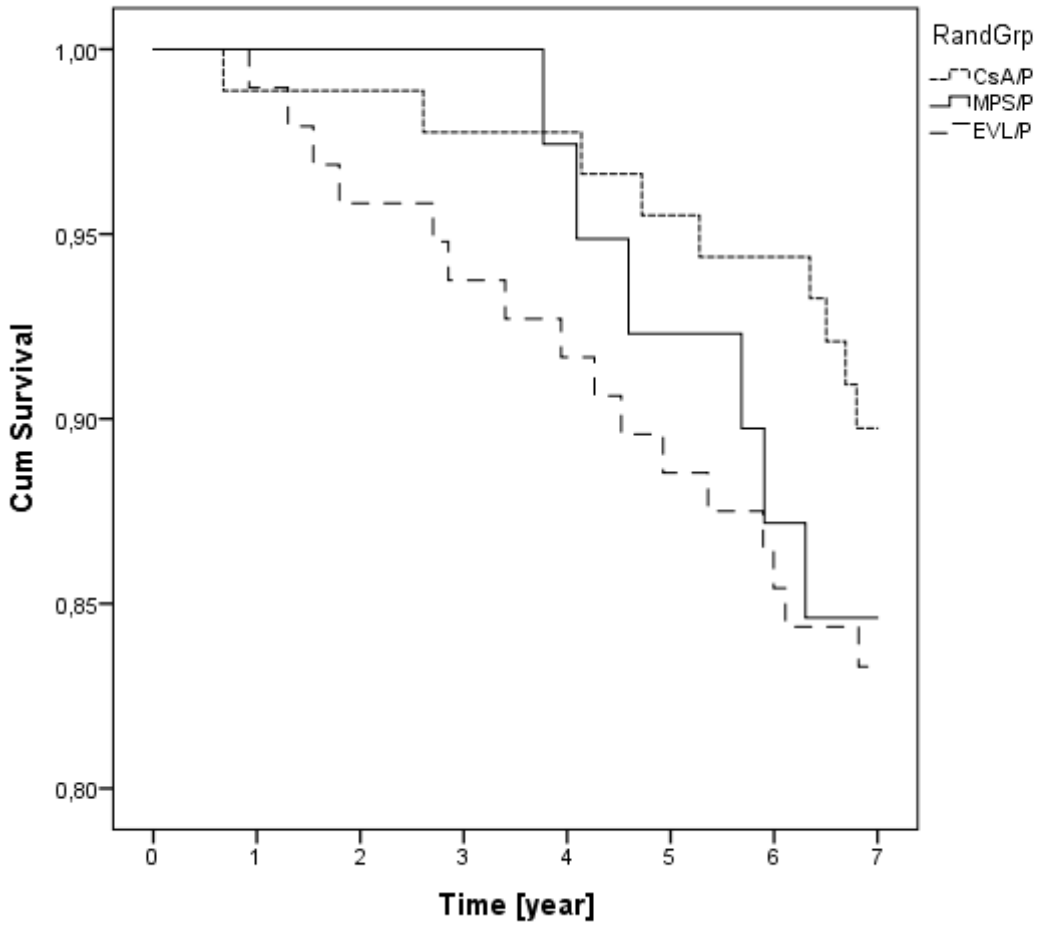
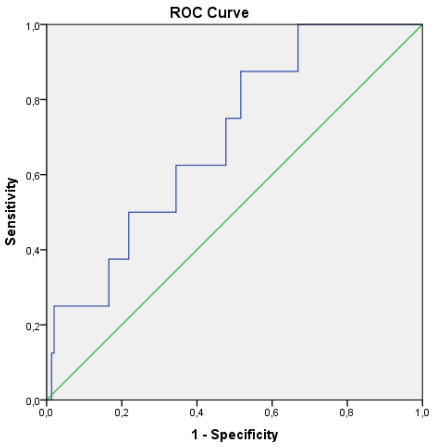
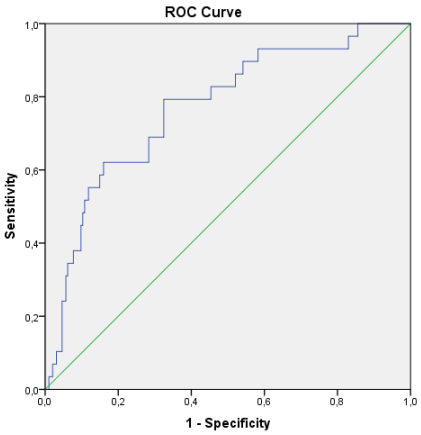


Figure 4: Receiver operating characteristics for major adverse cardiac event (A) and for mortality (B)



A



B